bio-protocol Preprint

Not Peer Reviewed
Ask a question online

CRISPR-Cas9 sgRNA design and construction

YH Yen-Lin Huang

Updated date: Nov 3, 2020

An abbreviated version of this protocol was published in eLIFE in Oct 2020

Collagen-rich omentum is a premetastatic niche for integrin α2-mediated peritoneal metastasis

DOI: 10.7554/eLife.59442

Detailed protocol

CRISPR-Cas9 sgRNA design and construction

Single guided RNAs (sgRNA) targeting exons 29–30 of *ITGA2* were designed using the web tool of UCSC Genome browser (https://genome.ucsc.edu/). The selected gRNAs were fulfilled the requirement as MIT guide specificity >60, high predicted cleavage efficiency > 95% (based on Doench et al., 2016 score). sgRNA1: GGTTCAGCTACTGAGCTCTG and sgRNA2: GAAGGTCTACAGCTCTAGAA respectively, were selected for gene editing of the transmembrane domain of *ITGA2* Exon 29-30). Single guided RNAs (sgRNA) targeting exons 26–29 of ITGA5 were sgRNA1: GTCCCGAGGAAGCAGAGCTG and sgRNA3: GCTAGGATGATGATCCACAG. pairs encoding 20nt targeted sequences with overhangs (both 5' and 3') from *BbsI* restriction site were annealed and cloned into either pSpCas9(BB)-2A-GFP (addgene, #PX458) or pSpCas9(BB)-2A-puro (addgene, #PX459). Constructs were transformed into DH5alpha *E. coli* strains and confirmed by Sanger DNA sequencing using U6-F primer. The detailed cloning procedure was provided from Zhang lab CRISPR plasmids from addgene https://media.addgene.org/cms/filer_public/e6/5a/e65a9ef8-c8ac-4f88-98da-3b7d7960394c/zhang-lab-general-cloning-protocol.pdf .

For construction of lentiCRISPRv2-based plasmid, the vector can be digested using *BsmBI* enzyme, and a pair of annealed oligos can be cloned into the single guide RNA scaffold. The oligos are designed based on the target site sequence (20bp) using the web tool of UCSC Genome browser (https://genome.ucsc.edu/). Synthesized desalted oligo 1 with 5' overhang-**CACC**G-N_{20} and oligo 2 with 5' overhang-**AAAC**-N_{20} were annealed and phosphorylated by T4 PNK enzymes in T4 ligation buffer at 37C for 30min and 95^{o}C 5 min. The temperature was ramped down to room temperature at 5^{o}C /min in a thermocycler. The detailed vector digestion and ligation protocol were adapted from the provided cloning protocols from addgene.
https://media.addgene.org/data/plasmids/52/52961/52961-attachment_B3xTwla0bkYD.pdf

References:

1. Improved lentiviral vectors and genome-wide libraries for CRISPR screening. Sanjana NE*, Shalem O*, Zhang F. Nature Methods (2014).
2. Genome-scale CRISPR-Cas9 knockout screening in human cells. Shalem O*, Sanjana NE*, Hartenian E, Shi X, Scott DA, Mikkelsen T, Heckl D, Ebert BL, Root D Doench JG, Zhang F (2014). Science, 343, 83-7. DOI: 10.1126/science.1247005.
3. Genome engineering using the CRISPR-Cas9 system. Ran FA, Hsu PD, Wright J, Agarwala V, Scott DA, Zhang F. Nat Protoc. 2013 Nov;8(11):2281-308. do 10.1038/nprot.2013.143. Epub 2013 Oct 24

How to cite:（Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used）

1. Huang, Y. (2020). CRISPR-Cas9 sgRNA design and construction. Bio-protocol Preprint. bio-protocol.org/prep600.
2. Huang, Y., Liang, C., Ritz, D., Coelho, R., Septiadi, D., Estermann, M., Cumin, C., Rimmer, N., Schötzau, A., Núñez López, M., Fedier, A., Konantz, M., Vlajnic, T., Calabrese, D., Lengerke, C., David, L., Rothen-Rutishauser, B., Jacob, F. and Heinzelmann-Schwarz, V.(2020). Collagen-rich omentum is a premetastatic niche for integrin α2-mediated peritoneal metastasis. eLIFE. DOI: 10.7554/eLife.59442

Copyright: Content may be subjected to copyright.